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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/686,789	10/17/2003	Francois Leblond	745969-128	7711

22204 7590 08/11/2005

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EXAMINER	
SINGH, SATYENDRA K	
ART UNIT	**PAPER NUMBER**
1651	

DATE MAILED: 08/11/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/686,789	LEBLOND ET AL.	
	Examiner	Art Unit	
	Satyendra K. Singh	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 October 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-36* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-36* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *06/07/2004 and 17 October 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *01/22/2004*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Specification

Claims 16, 31, and 35 have typographical mistakes. The term "islets of **Langerhans**" has been misspelled as "islets of Langherans" in each claim. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 36 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for treating experimentally-induced diabetes in mice, does not reasonably provide enablement for treating diabetes in a humans or other mammalian subjects. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with the claim.

While the claims of issued patents are interpreted in light of the specification, prosecution history, prior art and other claims, this is not the mode of claim interpretation to be applied during examination. During examination, the claims must be interpreted as broadly as their terms reasonably allow. (*In re American Academy of Science Tech Center, F.3d, 2004 WL 1067528 (Fed. Cir. May 13, 2004)*. It is only when the specification provides definitions for terms appearing in the claims that the

specification can be used in interpreting claim language. *(In re Vogel, 422 F.2d 438, 441,164 USPQ 619, 622 (CCPA 1970),* see MPEP **§** 2111.01).

The claim 36 is drawn to "a method for **treating diabetes in a subject**, said method comprising of: administering to said subject, an effective amount of the composition as defined in claim 32". The broadest reasonable interpretation of the term "treating" means treatment of a pre-existing diabetic condition in a subject using the claimed composition of the present invention. However, the instant specification defines the term "treating diabetes" in a much broader sense and also includes **prophylactic or preventive measures** in addition to the therapeutic treatments (see page 12, second paragraph, in particular) for which the specification is not enabled.

As discussed below, the specification disclosure does not enable one skilled in the art to practice the invention without an undue amount of experimentation.

At issue is whether or not the claimed method would function "for prophylaxis or prevention of diabetes". The nature of invention is such that it would require the administration of a composition comprising effective amounts of microencapsulated, living islets of Langerhans in a pharmacologically acceptable carrier to a subject *in vivo* in order to produce insulin in amounts sufficient to prevent onset of diabetes. The exemplification is drawn to a decrease in the severity of diabetes in streptozotocin-induced-diabetic mice model system as indicated by the percent of normoglycaemic animals obtained after intraperitoneal transplantation of microencapsulated cells (islets of Langerhans).

In re Fisher, 166 USPQ 18 indicates that the more unpredictable an area is, the more specific enablement is necessary in order to satisfy the statute. Since mice were used as animal model system to reduce diabetes, such animal model studies have not correlated well with *in vivo* clinical trial results in patients. Since the method of prophylaxis indices of administering to the animal a pharmaceutical composition comprising microencapsulated cells for the production of insulin hormone can be in fact, species and model dependent, it is not clear that reliance on the mice model studies accurately reflects the relative human efficacy of the claimed therapeutic strategy. In fact, the current assessment of islet transplantation research viewed from its immunological perspectives (see Inveradi et al, prior art review [X], see page 508, in particular) provides at best, a mixed outlook in terms of validation of the non-obese diabetic mice as clinically relevant model for islet transplantation to be applicable in human subjects. Inveradi et al [X] conclude that although tremendous progress has been witnessed in recent clinical trials, there is still the need to define safe and efficacious strategies to prevent islet rejection without chronic immunosuppression, attesting to the fact that immune responses of rodents such as mice are significantly different than humans (towards the grafted tissue or cells), and thus any correlation between the mouse diabetic model and the extrapolation of results obtained from animal studies to humans is still theoretical.

The specification does not adequately teach how to effectively prophylaxis of diabetes or reach any therapeutic end point in humans by administering such microencapsulated cells to a subject. The specification does not teach how to

extrapolate data obtained from mice studies to the development of effective *in vivo* mammalian (including humans) therapeutic prevention, commensurate in scope with the claimed invention. Therefore, it is not clear that the skilled artisan could predict the efficacy of the composition comprising the microencapsulated cells exemplified in the instant specification.

However, an effective preventive protocol for the prevention of diabetes in mammalian systems is subject to a number of factors, which enter the picture beyond simply the administration of a composition comprising microencapsulated living cells such as, islets of Langerhans. Demonstrating decrease in the severity of experimentally induced (using a pancreatic beta cell-destroying drug, streptozotocin) diabetes cannot alone support the predictability of the method for preventing said diabetes through administration of the appropriate composition. Zekorn et al (a review, Int. J. Artif. Organs, 1996, prior art [W]) clearly indicate factors that affect management of diabetes through bioartificial organs such as xenotransplantation procedures (including such microencapsulated islets of Langerhans; see abstract and pages 252-255, in particular). Zekorn et al [W] conclude that transplantation of encapsulated islets of Langerhans induces a variety of morphological reactions, among others (i.e. inflammation and fibrosis) as a result of a variety of donor and recipient related factors (biocompatibility and immune rejections), and factors such as long-term availability of the viable islets for insulin production, and therefore, warrants further investigation.

Therefore, there is insufficient guidance in the specification as to how to determine mammalian subjects such as humans in whom prevention is desired versus

in whom it is not desired. The specification also does not provide sufficient teaching as to how it can be assessed that prevention of diabetes in the mice was achieved after the administration of the composition comprising microencapsulated material such as claimed in the instant invention.

Reasonable correlation must exist between the scope of the claim and scope of the enablement set forth. In view of the quantity of the experimentation necessary the limited working examples, the nature of the invention, the state of the prior art, the unpredictability of the art, and the breadth of the claim, it would take undue trials and errors to practice the claimed invention.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 17, 18, 20-22 and 27-31 are rejected under 35 U.S.C. 102(b) as being anticipated by Chang et al (IDS).

Claim 17 is drawn to "a **semi-permeable microcapsule** comprising:

- **a bead suited to enclose a material**; and
- **a semi-permeable layer covering the bead**, said semi-permeable layer being made of a polycation cross-linking derivative **covalently inked** to the bead"

Chang et al (IDS) teaches a semi-permeable microcapsule comprising a bead (alginate-poly L-lysine gel core or droplets, as defined in the instant specification, page 6, second paragraph, in particular) suited to enclose a material (such as GH3 pituitary tumor cells; see prior art, introduction, page 118, second paragraph, in particular), a semi-permeable layer covering the bead (see Chang et al, abstract, materials and methods, Fig. 1, in particular), and the said semi-permeable layer being made of a polycation cross-linking derivative such as α- phenylcinnamylideneacetylated poly L-lysine used as a photosensitive poly(L-lysine) product (see Chang et al, materials and methods, page 119, and Fig. 1, in particular).

Claim 18 is drawn to "the microcapsule of claim 17, further comprising a **biocompatible layer covering said semi-permeable layer**, said biocompatible layer being **covalently linked** to the polycation cross-linking derivative of said semi-permeable layer" which is taught by the prior art Chang et al (IDS). Chang et al (IDS) teach a biocompatible layer made of a "(short-chain alginate)-co-MPEG" block copolymer covering the semi-permeable layer and being covalently cross linked to it (see Chang et al, abstract; page 118-119, and Fig. 1, in particular) using the polycation cross-linking derivative (such as α- phenylcinnamylideneacetylated poly L-lysine).

Claims 20-22 are drawn to "the microcapsule of claim 18, wherein the bead and the biocompatible layer comprise a negatively-charged compound; wherein the compound is a hydrogel; and wherein the hydrogel is alginate" which is taught by the prior art, Chang et al (IDS). Chang et al teach a microcapsule such as claimed wherein the microcapsule bead and the biocompatible material comprise a negatively charged

compound which is a hydrogel such as alginate (see prior art, abstract; materials and methods; page 118-120, in particular).

Claims 27 and 28 (depends from claim 27) are drawn to "the microcapsule of claim 17, wherein said microcapsule allows passage of molecules with a defined viscosity radius; and wherein said viscosity radius is equal or inferior to about 2.7 nm" which is taught by the prior art, Chang et al (see abstract, and pages 120-121, sections, "permeability test" and "permeability of the microcapsules", respectively, in particular). Prior art teaches the fact that semi-permeable microcapsules are found to be quite permeable to Cytochrome C (12 kDa), were almost impermeable to bovine serum albumin (66 kDa), and had an intermediate permeability to Myoglobin (19 kDa).

Although the Chang et al (IDS) does not use the same criteria to evaluate the permeability of microcapsules as the current application (using molecular weight cut-off in a size exclusion chromatography; see instant specification, example 5, page 18, in particular), the use of free diffusion of the homogeneously purified protein molecules (used by Chang et al as molecular weight markers) into the microcapsules of the prior art will inherently depend on the defined viscosity radius of the individual protein molecules (as taught by the applicants in the instant specification for various dextran and protein molecular weight markers used for the size exclusion chromatography in example 5, in particular) and since the prior art demonstrates the permeability of Cytochrome C and Myoglobin molecules (having a viscosity ratio of less than 2.7 nm, based on their molecular weights being less than carboxypeptidase; as supported by the instant specification on page 19) into the microcapsules, albeit under a different but

analogous experimental conditions where protein molecules are allowed to diffuse freely into the microcapsule pores, the limitation claimed are inherently met by the referenced invention.

Claims 29-31 are drawn to limitations in the claimed invention of claim 17, which are inherently taught by the prior art Chang et al (IDS), and which do not further impart any structural limitation to the base claim 17, and are thus anticipated by Chang et al (IDS).

Claim 29 is drawn to "the microcapsule of claim 17, wherein said material is living cells" is taught by the prior art (see, introduction, second paragraph, page 118, and references therein, in particular) Chang et al (IDS) wherein the entrapment of living cells such as cultured GH3 pituitary cells within the alginate-poly L-lysine microcapsules is disclosed.

Claims 30-31 (depend from claim 29) are drawn to "the microcapsule of claim 17, wherein the living cells are insulin-producing cells; and wherein said insulin-producing cells are comprised in islets of Langerhans". The limitations of claim 30 and 31 are inherently met by the prior art reference (Chang et al, IDS) as they teach the microcapsule such as claimed **suitable for encapsulation** of cells such as GH3 pituitary tumor cells, and suggest the use of such microcapsules in xenotransplantation (see page 125, second paragraph, in particular) of tissue materials for human therapy.

Since only suitability, no actual islets encapsulation is required, one skilled in the art would reasonably conclude that if the microcapsules of the referenced invention

(Chang et al, IDS) are suitable for encapsulation of GH3 cells, then they are also suitable for islets of Langerhans because both are animal-derived cells capable of secreting polypeptide hormones under appropriate culture and biological conditions (see prior art, Ambion [U]), and will be no different in regards to their encapsulation in the semi-permeable microcapsules such as taught by the prior art, Chang et al (IDS).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 32-35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chang et al (IDS) as applied to claims 17-18, and 20-22 and 27-31 above, and further in view of Hubbell et al (U.S. Patent 5,801,033 [A]).

Claims 32-35 are drawn to "a **pharmaceutical composition** comprising:

- a plurality of **semi-permeable microcapsules**, each one being as defined in claim 17 and, each one of said microcapsules enclosing a material; and
- a pharmaceutically acceptable **carrier**;
- wherein said material is **living cells**;
- wherein said living cells are **insulin-producing cells**; and wherein said insulin-producing cells are comprised in **islets of Langerhans**".

Chang et al (IDS) teach a semi-permeable microcapsule (as discussed, supra) comprising a bead (alginate-poly L-lysine gel core or droplets, as defined in the instant specification, page 6, second paragraph, in particular) suited to enclose a material (such as biological materials and cells, see prior art, introduction, page 118, second paragraph, in particular), a semi-permeable layer covering the bead (see prior art, abstract, materials and methods, Fig. 1, in particular), and the said semi-permeable layer being made of a polycation cross-linking derivative such as α-phenylcinnamylideneacetylated poly L-lysine used as a photosensitive poly(L-lysine) product (see prior art, materials and methods, page 119, and Fig. 1, in particular). Chang et al teach a biocompatible layer made of a "(short-chain alginate)-co-MPEG" block copolymer covering the semi-permeable layer and being covalently cross-linked to it (see Chang et al; abstract, page 118-119, and Fig. 1, in particular). Chang et al (IDS) also teach a microcapsule such as claimed wherein the microcapsule bead and the

biocompatible material comprise a negatively charged compound which is a hydrogel such as alginate (see abstract, materials and methods, page 118-120, in particular).

Chang et al (IDS) suggest the use of such microcapsules (as claimed in claim 17) (owing to their superior stability and biocompatibility, see prior art, introduction; and page 125, second paragraph, in particular) in xeno-transplantation for obtaining a long-term survival of xenograft tissue for human therapy. However, a pharmaceutical composition comprising a pharmaceutically acceptable carrier and plurality of such semi-permeable microcapsules each enclosing biological material such as living cells, insulin producing cells, and islets of Langerhans, is not explicitly taught by Chang et al.

Prior art, Hubbell et al [A] teach a composition comprising semi-permeable microcapsules in a pharmaceutically acceptable carrier such as HEPES buffered saline (see column 19, example 10 and 32, in particular), each enclosing biological material such as living cells which produce insulin (islets of Langerhans isolated from human pancreas; see Hubbell et al, column 10, biological materials; and examples 5, 6, 7, and 9, in particular).

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to encapsulate biological materials including living cells producing insulin such as islets of Langerhans as taught by Hubbell et al [A] in the semi-permeable microcapsules comprising alginate-poly L-lysine-alginate structure as taught by the prior art Chang et al (IDS) in order to obtain a pharmaceutical composition in a pharmaceutically acceptable carrier such as claimed in claims 32-35.

The person of ordinary skill in the art would have been motivated to make such a composition as the prior art Chang et al (IDS) explicitly suggests the use of such microencapsulated materials for xeno-transplantation and human therapy owing to their increased mechanical strength, stability, and long term survival of the enclosed cells or biological materials, and the improved biocompatibility of the microcapsules (see Chang et al, introduction, page 118; and discussion, page 125, second paragraph; and references therein, in particular).

One of ordinary skill in the art would have had a reasonable expectation of success when making the pharmaceutical composition comprising a pharmaceutically acceptable carrier and micro-encapsulation of insulin producing cells such as pancreatic islets of Langerhans as taught by Hubbell et al [A] using the semi-permeable microcapsules as taught by Chang et al (IDS) because the practice and composition of such microcapsules enclosing living cells producing insulin, such as islets of Langerhans has been explicitly taught by Hubbell et al [A]. In fact, Hubbell et al teach the use of such composition along with the benefits which accrue from the microencapsulation of biological materials (see column 8, second and third paragraph; column 10, biological materials; column 12, last paragraph; and examples, in particular).

Thus the invention as a whole would have been *prima facie* obvious to one skill in the art at the time the invention was made.

Claim 36 is rejected under 35 U.S.C. 103(a) as being unpatentable over Chang et al (IDS) and Hubbell et al (U.S. Patent 5,801,033 [A]) as applied to claims 17-18, 20-22, and 27-35 above, and further in view of Lamberti (U.S. Patent 5,827,707, [B]).

Claim 36 is drawn to " a method of treating diabetes in a subject, said method comprising the step of: administering to said subject, an effective amount of the composition as defined in claim 32".

Since the claimed method is enabled for treating only the **experimentally-induced diabetes in mice** (see enablement discussion, supra) as exemplified in the instant specification, the examination of the claimed invention hereafter, is therefore limited to the claimed aspects directly related to such method only.

As discussed (supra), Chang et al (IDS) teach a semi-permeable microcapsule suitable for use in xeno-transplantation for obtaining a long-term survival of xenograft tissue for human therapy. Hubbell et al [A] teach a composition comprising semi-permeable microcapsules in a pharmaceutically acceptable carrier such as HEPES buffered saline each enclosing biological material such as living cells which produce insulin (islets of Langerhans isolated from human pancreas; see prior art, column 10, biological materials; and examples 5, 6, 7, and 9, in particular).

However, a method of treating experimentally-induced diabetes in mice comprising the steps of administering to a said subject, an effective amount of the composition (as defined in claim 32) is not explicitly disclosed by the prior arts Chang et al (IDS) and Hubbell et al [A].

Lamberti [B] teaches a method for manufacturing minimal volume capsules containing biological materials that can be potentially used for xenotransplantation and other biomedical procedures (see abstract, summary of the invention, column 1 and example 5, in particular). Lamberti [B] teaches an *in vivo* method of treating streptozotocine (STZ)-induced diabetic mice (as exemplified by the instant specification, see page 24, example 7, in particular) using the microencapsulated islets of Langerhans from porcine sources.

Therefore, it would have been obvious to a person of ordinary skill in the art at the time this invention was made to use the composition of semi-permeable microcapsules comprising encapsulated islet cells as taught by the prior art Chang et al (IDS) and Hubbell et al [A] for the treatment of STZ-induced diabetes in experimental mice model system in order to evaluate the efficacy and the viability of the encapsulated cells for the production of insulin in vivo.

The person of ordinary skill in the art would have been motivated to use an experimental model system such as taught by Lamberti [B] to evaluate the effectiveness and the long-term use of such microencapsulation compositions containing living cells (such as islets of Langerhans), because the Lamberti [B] explicitly discloses the benefits and use of such microcapsules in providing the potential for treatment (in future) of insulin-dependent diabetes mellitus (IDDM) in humans through transplantation of insulin-producing cells or cell aggregates, and timed release or long-term delivery of drugs to an animal (see column 1, first paragraph, in particular).

One of ordinary skill in the art would have had a reasonable expectation of success when using such method for treating STZ-induced diabetes in mice model system as taught by the prior art [B] with the microcapsule composition containing islet cells as taught by the prior arts, Chang et al and Hubbell et al [A], because the practice and use of such a method is explicitly taught by the prior art Lamberti [B] (see discussion, supra).

Thus the invention as a whole would have been *prima facie* obvious to one skill in the art at the time the claimed invention was made.

Claims 19 and 23-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chang et al (IDS) as applied to claims 17, 18, and 20-22, and 27-31 above, and further in view of Pierce Biotechnology, Inc. [V].

Claim 19 and 23-25 are drawn to "the microcapsule of claim 17, wherein said polycation cross-linking derivative is a polycation covalently linked to a photoactivable cross-linking agent, said agent comprising:

- a **N-hydroxysuccinimide ester group**; and a **phenyl azide group**;
- - wherein the polycation is poly L-lysine;
- - wherein the photoactivable cross-linking agent is N-5-azido-2-nitrobenzoyloxysuccinimide (**ANB-NOS**); and
- - wherein the polycation is **poly L-lysine** and the photoactivable cross-linking agent is N-5-azido-2-nitrobenzoyloxysuccinimide (**ANB-NOS**)".

Chang et al (IDS) teach a semi-permeable microcapsule (as discussed, supra) comprising a bead (alginate-poly L-lysine gel core) suited to enclose biological materials such as cells, having a semi-permeable layer covering the bead and the said semi-permeable layer being made of a polycation cross-linking derivative such as α-phenylcinnamylideneacetylated poly L-lysine used as a photosensitive poly (L-lysine) product covalently linked to the bead (see discussion, supra).

Chang et al (IDS) teach the microcapsules covered with semi-permeable layer being made of a polycation such as is poly L-lysine. However, the limitation polycation covalently linked to photoactivable cross-linking agent comprising a N-hydroxysuccinimide ester group; and a phenyl azide group is not disclosed.

Use of photoactivable cross linkers has been a common knowledge to one skilled in the art, and is evident by the fact that a commercial company such as Pierce Biotechnology Inc. (see, prior art [V]) sales as well as teaches the use of such photoactivable crosslinkers (see Pierce Doc no. 0635, and references therein) as "heterobifunctional photoreactive cross linkers" which consist of a N-hydroxysuccinimide ester group and a phenyl azide group as reactive groups (for example, ANB-NOS and Sulfo-SANPAH among others, as disclosed in the instant specification, example 1, page 14, first paragraph, in particular). Pierce Biotech. Inc. [V] teaches the usefulness of ANB-NOS such as- it requires a broader wavelength of UV light (in the range of 300-460 nm), can be used at slightly basic pH range such as pH 7.0 to 9.0 (which encompasses the cellular pH requirements for biocompatibility), and cites several

examples where ANB-NOS has been used to photocrosslink proteins and other macromolecules (see prior art [V], and references therein).

It would have been obvious to a person of ordinary skill in the art at the time this invention was made to substitute the photosensitive Cross linker, α-phenylcinnamylideneacyl chloride used in the prior art (chang et al, IDS) with the heterobifunctional, photoreactive cross linkers such as ANB-NOS as taught by Pierce Biotech. Inc.[V], in order to achieve the superior mechanical strength and stability of the microcapsules for xenotransplantation purposes.

The person of ordinary skill in the art would have been motivated to change the photoactivable cross-linking agent to one having a N-hydroxysuccinimide ester group and a phenyl azide group such as ANB-NOS because Pierce Biotech. Inc. [V] teaches the benefits of using such a photoreactive cross linking agent that is a heterobifunctional, relatively non-specific (therefore, useful for extensive cross linking between polysaccharide such as alginate and a polycationic protein having free epsilon amino groups, such as poly L-lysine; and can be easily quenched by water molecules in aqueous solutions), membrane permeable (and thus effective for quick and extensive crosslinking within the bead as well as between the bead and the semi-permeable layer of the polycation used) cross linker and has been used for inter- and intra-molecular cross linking of proteins and other macromolecular structures (see prior art, document no. 0635 and references therein, in particular).

One of ordinary skill in the art would have had a reasonable expectation of success when substituting with the cross linker as taught by the Pierce Biotech. Inc. [V]

because the prior art discloses the usefulness of the photoreactive heterobifunctional cross linker, ANB-NOS in quick and extensive crosslinking of proteins through their free amino groups to other macromolecular structures, and thus provides the basis for its widespread use as a preferred photoreactive cross linker. The person of ordinary skill in the art would have been motivated to use ANB-NOS in place of α-phenylcinnamylideneacyl chloride as a photocrosslinking agent in order to provide both superior mechanical strength and chemical stability to the semi-permeable microcapsules such as taught by the prior art Chang et al.

The limitation in claim 26 "wherein the poly L-lysine and the ANB-NOS are in a 1:20 ratio" is an obvious matter of routine optimization in view of the teachings of the prior art [V] as evidenced by the fact that the prior art provides a detailed and explicit technical information as to how to use such heterobifunctional crosslinkers including an optimal pH range, concentration of reactants and the range of the final concentration of the crosslinker used (such as use of "a 20- to 50-fold molar excess of the crosslinker").

Thus the invention as a whole would have been *prima facie* obvious to one skill in the art at the time the invention was made.

Claims 1-2, 4-9, and 14-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chang et al (IDS) as applied to claims 17, 18, and 20-22 and 27-35 above, and further in view of Hubbell et al (U.S. Patent 5,801,033 [A]).

Claim 1 is drawn to "**a method for microencapsulating a beaded material** said method comprising the steps of:

a) providing a material enclosed within a bead to obtain a beaded material;

b) covering the beaded material with a semi-permeable layer made of a polycation cross-linking derivative, to obtain a product; and

c) covalently linking the beaded material to the semi-permeable layer".

Claims 14-16 (depend from claim 1) are drawn to "the method of claim 1, wherein said beaded material is beaded living cells; wherein said living cells are insulin producing cells; and wherein said insulin-producing cells are comprised in islets of Langerhans"

Chang et al (IDS) teach a semi-permeable microcapsule comprising a bead (calcium alginate core) suited to enclose biological materials such as cells, having a semi-permeable layer covering the bead and the said semi-permeable layer being made of a polycation cross-linking derivative such as α- phenylcinnamylideneacetylated poly L-lysine used as a photosensitive poly (L-lysine) product covalently linked to the bead (see, discussion, supra).

However, the method for microencapsulating a beaded material comprising a step of "providing a material enclosed within a bead" is not explicitly disclosed by Chang et al.

Hubbell et al [A] teach a method of microencapsulating beaded material (see abstract, Fig. 2 and 3, column 7 and 8, and example 32, in particular) such as living cells (pancreatic islets of Langerhans) enclosed in alginate-poly (L-lysine) core beads or

droplets stabilized by free radical polymerization using variety of procedures on either coated or uncoated biological material.

It would have been obvious to a person of ordinary skill in the art at the time this invention was made to use the method for microencapsulation of beaded material such as to enclose living cells as taught by Hubbell et al [A] using the semi-permeable microcapsules of the prior art invention as taught by Chang et al (IDS).

The person of ordinary skill in the art would have been motivated to use the method for encapsulation of islets of Langerhans using the microcapsules as taught by Chang et al, because the prior art (Hubbell et al [A]) teaches the benefit of such microencapsulation procedure for biological materials such as mammalian tissues and/or cells, sub-cellular organelles, and other isolated sub-cellular components including the goal for long term survival of cells which are to be used for the production of desired products such as proteins (see prior art [A], column 10, section biological materials, in particular).

One of ordinary skill in the art would have had a reasonable expectation of success when using the method for microencapsulation for beaded material such as living cells as taught by Hubbell et al, as it explicitly teaches the encapsulation of pancreatic islet of Langerhans (which could be used for producing insulin hormone). Hubbell et al [A] also teach the fact that the biological material can be first enclosed in a structure (such as beads, droplets, etc.) and then can be further crosslinked or modified with suitable coating structures (see column 7, 8, and 10, in particular).

Thus the invention as a whole would have been *prima facie* obvious to one skill in the art at the time the invention was made.

Claim 2 is drawn to "the method of claim 1 comprising, after step b), a step of covering the product of step b) with a biocompatible layer; and wherein, in step c), said semi-permeable layer of the product of step b) is further covalently linked to said biocompatible layer".

Claims 4-6 are drawn to "the method of claim 2, wherein step c) of covalently linking said beaded material to said semi-permeable layer or said step of covalently linking said semi-permeable layer of the product of step b) to both the beaded material and the biocompatible layer, is obtained by a step of exposing the polycation cross-linking derivative of the semi-permeable layer to a predetermined dose of light; and wherein the light is UVA light; and wherein the predetermined dose of light is at least about 2 kJ/m^2 and less than about 23 kJ/m^2".

Claims 7-9 are drawn to "the method of claim 2, wherein the bead and the biocompatible layer comprise a negatively-charged compound; wherein the negatively-charged compound is a hydrogel; and wherein the hydrogel is alginate".

Prior art, Chang et al (IDS) teach the alginate-photosensitive poly L-lysine microcapsule which are further coated with a biocompatible layer (negatively charged compound or a hydrogel) of alginate (short-chain)-co-MPEG block copolymer covalently linked in order to impart good mechanical strength and biocompatibility (see prior art, abstract, introduction, and materials and methods, in particular). Chang et al teach the

step of exposing the polycation cross-linking derivative of the semi-permeable layer to a predetermined dose of light such as irradiating for 4 min with a 400W high-pressure mercury lamp, which emits UVA light (see Chang et al, page 120, first paragraph, and references therein, in particular).

Hubbell et al [A] teach a method of microencapsulation of biological material such as living cells using alginate-poly L-lysine core wherein the radiation used to initiate the polymerization of the polycation is either longwave UV or visible light (see Hubbell et al, column 12, second paragraph, in particular) in the range of 320-900 nm. Hubbell et al explicitly disclose that the light can be provided from any appropriate source such as mercury lamp, longwave UV lamp, etc.

In view of teachings from the prior arts, Chang et al (IDS) in view of Hubbell et al [A], it would have been obvious to a person of ordinary skill in the art at the time this invention was made to covalently link the semi-permeable polycation layer covering the beaded material with a biocompatible layer of alginate or its derivatives, wherein the covalent linking of the semi-permeable layer is obtained by a step of exposing the photosensitive polycation (poly L-lysine) derivative, as taught by Chang et al to a predetermined dose of UVA light.

The person of ordinary skill in the art would have been motivated to include such a step of using a predetermined dose of UVA light to crosslink the semi-permeable layer to the beaded material in the method of microencapsulation as disclosed by Hubbell et al, because both prior arts teach the use of such a light source in order to avoid damage

to the living cells enclosed in the microcapsules of the referenced invention (see Hubbell et al [A], column 6, third paragraph, in particular).

The limitation in claim 6 that the predetermined dose of light is at least about 2 kJ/m^2 and less than about 23 kJ/m^2 is a clearly matter of routine optimization by one skilled in the art as evidenced by the disclosure of technical details in the prior art (Chang et al, supra) such as the use of 400W high-pressure mercury lamp for 4 min (i.e. a predetermined dose of UVA light) to induce photopolymerization of the photosensitive poly L-lysine derivative covering the beaded material of the referenced invention.

One of ordinary skill in the art would have had a reasonable expectation of success when adding an extra layer of the biocompatible alginate layer and covalently crosslinking the photosensitive semi-permeable poly L-lysine layer to the beaded material, as the combined teachings of the prior arts, Chang et al (IDS) and Hubbell et al [A], clearly teach the steps required to devise such a method of microencapsulation of biological material as claimed. It would have been obvious to use longwave UVA light at a predetermined dose level/range in order to avoid serious damage to the biological material enclosed especially in lieu of the fact that long-term survival of the enclosed tissue or cells is deemed critical for xenotransplantation purposes, and has been appropriately taught by the said prior arts.

Thus the invention as a whole would have been *prima facie* obvious to one skill in the art at the time the invention was made.

Claims 3 and 10-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chang et al (IDS) and Hubbell et al (U.S. Patent 5,801,033 [A]) as applied to claims 1-2, 4-9, 14-18, 20-22, and 27-35 above, and further in view of Pierce Biotechnology, Inc. [V].

Claim 3 is drawn to "the method of claim 1 comprising, prior to step b), a step of covalently linking a polycation to a photoactivable cross-linking agent to obtain the polycation cross-linking derivative of step b), said photoactivable crosslinking agent comprising: a N-hydroxysuccinimide ester group; and a phenyl azide group".

Claim 10-12 (depend from claim 3) and claim 13 are drawn to "the method of claim 3, wherein the polycation is poly L-lysine; wherein the photoactivable crosslinking agent is N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOS); wherein the polycation is poly L-lysine and the photoactivable cross-linking agent is N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOS); and wherein the poly L-lysine and the ANB-NOS are mixed together in a 1:20 ratio".

Hubbell et al [A] teach a method of microencapsulating beaded material (see abstract, Fig. 2 and 3, column 7 and 8, and example 32, in particular) such as living cells (pancreatic islets of Langerhans) enclosed in alginate-poly (L-lysine) core beads or droplets stabilized by free radical polymerization using variety of procedures on either coated or uncoated biological material (see discussion, supra).

Chang et al (IDS) teach a semi-permeable microcapsule comprising a bead (alginate-poly L-lysine gel core) suited to enclose biological materials such as cells,

having a semi-permeable layer covering the bead and the said semi-permeable layer being made of a polycation cross-linking derivative such as α-phenylcinnamylideneacetylated poly L-lysine used as a photosensitive poly (L-lysine) product covalently linked to the bead (see discussion, supra).

Chang et al (IDS) teach the microcapsules covered with semi-permeable layer being made of a polycation such as is poly L-lysine. However, the limitation polycation covalently linked to photoactivable cross-linking agent comprising a N-hydroxysuccinimide ester group; and a phenyl azide group; wherein such a photoactivable cross-linking agent is ANB-NOS, is not disclosed.

Pierce Biotechnology Inc.,(see, Prior art [V]) teaches the use of such photoactivable crosslinkers (see prior art [V], Doc no. 0635, and references therein) as "heterobifunctional photoreactive cross linkers" which consist of a N-hydroxysuccinimide ester group and a phenyl azide group as reactive groups (for example, ANB-NOS and Sulfo-SANPAH among others, as disclosed in the instant specification, example 1, page 14, first paragraph, in particular). Prior art teaches the usefulness of ANB-NOS such as its requirement for a broader wavelength of UV light (in the range of 300- 460 nm), can be used at slightly basic pH range such as pH 7.0 to 9.0 (which encompasses the cellular pH requirements for biocompatibility), and cites several examples where ANB-NOS has been used to crosslink proteins and other macromolecules (see discussion, supra).

It would have been obvious to a person of ordinary skill in the art at the time this invention was made to substitute the photosensitive Cross linker, α-

phenylcinnamylideneacyl chloride used in the method step of covalently linking a poly L-lysine to a photoactivable crosslinking agent (chang et al) with the heterobifunctional, photoreactive cross linker such as ANB-NOS as taught by prior art [V] in order to achieve superior mechanical strength and stability for the microcapsules enclosing a beaded material (such as living cells) for xenotransplantation purposes using the microencapsulation procedure as taught by Hubbell et al.

The person of ordinary skill in the art would have been motivated to change the photoactivable cross-linking agent to one having a N-hydroxysuccinimide ester group and a phenyl azide group such as ANB-NOS because prior art [V] teaches the benefits of using such a photoreactive cross linking agent that is a heterobifunctional, relatively non-specific (therefore, useful for extensive cross linking between polysaccharide such as alginate and a polycationic protein having free epsilon amino groups, such as poly L-lysine; and can be easily quenched by water molecules in aqueous solutions), membrane permeable (and thus effective for quick and extensive crosslinking within the bead as well as between bead and the semi-permeable layer of the polycation used) cross linker and has been used for inter- and intra-molecular cross linking of proteins and other macromolecular structures (see prior art, document no. 0635 and references therein, in particular).

One of ordinary skill in the art would have had a reasonable expectation of success when substituting the cross linker as taught by the prior art [V] because the prior art discloses the usefulness of the photoreactive heterobifunctional cross linker, ANB-NOS in quick and extensive crosslinking of proteins through their free amino

groups to other macromolecular structures, and thus provides the basis for its widespread use as a preferred photoreactive cross linker. The person of ordinary skill in the art would have been motivated to use ANB-NOS in place of α-phenylcinnamylideneacyl chloride as a photocrosslinking agent in order to provide both superior mechanical strength and chemical stability to the semi-permeable microcapsules such as taught by the prior art Chang et al.

The limitation in claim 13 "wherein the poly L-lysine and the ANB-NOS are mixed together in a 1:20 ratio" is an obvious matter of routine optimization in view of the teachings of the prior art [V] as evidenced by the fact that the prior art provides a detailed and explicit technical information as to how to use such heterobifunctional crosslinkers including an optimal pH range, concentration of reactants and the range of the final concentration of the crosslinker used (such as use of "a 20- to 50-fold molar excess of the crosslinker").

Thus the invention as a whole would have been *prima facie* obvious to one skill in the art at the time the invention was made.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Satyendra K. Singh whose telephone number is 571-272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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